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New agents for the treatment of infarcted myocardium

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ABSTRACT

Local delivery of angiogenic growth factors for the treatment of myocardial ischemia has been well documented in various animal models, and clinical trials are now in progress. Our strategy was radically different, based on selective protection of some of the growth factors naturally present within the injured tissue. This protection was obtained by applying a chemically defined substitute for Dextran called RGTA11 (for ReGeneraTing Agent). RGTA is a family of agents, which has properties mimicking those of heparan sulfates toward heparin-binding growth factors (HBGF) and which stimulate tissue repair and protection. Indeed, we have previously shown that RGTA prevents most of the damage resulting from acute skeletal muscle ischemia [*FASEB J.* (1999) **13**, 761–766]. We now show that the same agent can be used for the treatment of myocardial infarction. Acute myocardial infarction was induced in pigs by ligation of the left circumflex artery. One hour later, a single injection of 10 μg of RGTA11 was made in the center of the infarcted area. Three weeks later we observed *1*) recovery of 84% of the initial left ventricular ejection fraction (only 55% in saline-treated controls), *2*) an almost 50% reduction in the infarct size, *3*) a reduction in fibrotic tissue formation, *4*) significant preservation of myocytes, and *5*) an increase in the number of blood vessels. The treatment of ischemic heart disease with RGTA would have clear advantages over other therapies such as growth factor, gene, or cell transplants, based on a stable, simple, and easy-to-develop chemical product.

Key words: ischemia • heart • RGTA • heparan sulfate mimetic

Ischemia of cardiac muscle induces myocardial infarction and results in an irreversible loss of cardiac myocytes and necrosis. Any rescue strategy after acute myocardial infarction must protect cardiomyocytes against hypoxia and enhance blood delivery. In a recent study we found that RGTA synthetic polymers, designed to mimic the stabilizing and protecting properties of heparan sulfates toward heparin-binding growth factors (or HBGF) (1), prevented some of the damage resulting from muscle ischemia (2). These polymers were also able to stimulate tissue repair when applied at the site of the injury, and enhanced healing was subsequently observed in

various *in vivo* models including skin (3), bone (4), colon (5), cornea (6), as well as deinnervated and crushed *Extensor Digitorum Longus* and *Soleus* muscles (7, 8).

The RGTA used in these studies were Dextran derivatives containing defined amounts of substituted carboxymethyl, benzylamide, and sulfonate groups. *In vitro*, RGTA can interact with and protect various HBGFs such as FGF and TGFβ against proteolytic degradation (1, 5). RGTA are devoid of significant anticoagulant and anticomplement activities and are potent inhibitors of human leukocyte elastase (9), plasmin (10), and heparanases (Vlodavski, I. and Barritault, D., unpublished results). On the basis of these *in vitro* properties, RGTA are believed to enhance the bioavailability of HBGFs *in vivo*.

Recent studies have demonstrated that injections of growth factors, such as FGFs (11, 12) and VEGF (13), or their genes, into ischemic myocardium improves myocardial function. All these angiogenic factors promote collateral vascularization (14). Intrapericardial injection reduced the size of the injured zone and promoted functional recovery of the heart in a canine model of myocardial infarction (15), underlining the importance of the choice of animal model and of preexisting collateral vascularization, as found in dogs.

With this in mind, we examined whether RGTA combining the twin properties of tissue protection against anoxia and HBGF protection could improve the healing process and the recovery of left ejection fraction after myocardial infarction in pigs, an animal with similar vascularization to humans.

MATERIALS AND METHODS

RGTA preparation and *in vitro* testing

RGTA11 is a Dextran derivative obtained by controlled and sequential substitutions on the glucose residues of a batch of Dextran T40, as previously described (16). The percentages of substitutions were 69, 2.5, and 36.5% for the carboxymethyl, carboxymethylbenzylamine, and carboxymethyl-benzylamide sulfonate and sulfate groups, respectively. The anticoagulant activity was 3.5 IU/mg (compared with heparin, usually at ~170/180 IU/mg).

Coronary occlusion

Large, white male pigs (n=14) of the same age and weight (32±2 kg) were used. Twice a week, one control and one treated animal, which were randomly chosen in order to level the differences of initial extent and severity of ischemia, were operated on. Animals were premedicated with ketamine hydrochloride (5–10 mg/kg i.m.). General anaesthesia was induced with sodium thiamylal (10–20 mg/kg i.v.); the animals were then intubated and anaesthesia was maintained with halothane (0.5–1.5 vol%). Occlusion of the left circumflex artery was chosen, as there was no mortality associated with the resulting infarction. Five animals were used for training in order to find the best experimental conditions; therefore, none of the animals were excluded from the analysis once the experiment proper started. Furthermore, as dissection of tissue surrounding the left circumflex coronary artery (LCX) induced spasms that may alter the flow if allowed to occur for too long, thereby inducing preconditioning, rapid, permanent double ligation was performed.

The animals were not reperfused. After pericardiotomy, the LCX was carefully exposed 3–5 cm from its origin and acute double ligation was performed 3 cm from its origin. Coronary occlusion was confirmed by selective coronary angiography 1 h after ligation and by the visible epicardial cyanosis. In each pig, the total volume (100 μl) of saline (controls) or RGTA (100 μg/ml) was injected into the myocardium at six points around the infarction (visualized by epicardial cyanosis), and directly into the infarct site, using a Hamilton syringe with a specific sharp and flexible needle (N-50 type B; Ito, Fuji, Japan) chosen to minimize physical damage to muscle fibers (2). The pericardium and the incision were closed in layers, and the chest was evacuated. Blood temperature of all pigs was maintained between 36.5 and 37°C throughout the experiment. During coronary artery occlusion, multiple premature ventricular contractions were treated with bolus injections of lidocaine (20 mg/kg). All pigs were treated with 20 mg/kg amoxicillin after surgery and for up to 7 days. All animals received care in accordance with institutional guidelines.

Hemodynamic parameters

ECG, heart rate, right atrial pressure, pulmonary artery pressure, pulmonary capillary wedge pressure, and arterial pressure were recorded at baseline, 10 min, 60 min, 90 min, and 3 weeks after LCX ligation. Left ventricular end-diastolic pressure was recorded before left ventriculography on a Hewlett Packard M1117A. Cardiac output was determined by thermodilution method (10 ml 0.9% NaCl, 0–4°C) at the same time points as arterial pressure. Systemic vascular resistance, pulmonary vascular resistance, and stroke work index were calculated by the use of the given indexes above. Left ventricular and diastolic pressures (LVEDP) were measured using an 8F-pigtail catheter. Selective left coronary angiography, used to estimate the coronary anatomy, was performed using a 6F catheter. Then, left ventriculography was performed to measure the baseline ejection fraction. Left ventricular ejection fractions (LVEF) were determined from single-plane left ventriculograms by a trained technician who was blinded to the treatment assignment. Ejection fractions and simultaneous left ventricular end-diastolic (LVEDV), end-systolic (LVESV), and systolic ejection volumes (LVSEV) were calculated by using the length-area method with a computer analysis package (Phoenix 80286 ROM BIOS Plus Version 3.10). Ejection fractions were measured at baseline, 60 min, and 3 weeks after coronary ligation.

Determination of infarct size

After three weeks, the heart was exposed by median sternotomy, the ascending aorta was dissected and clamped, and 100 ml of cold hyperkalemic solution (40 mEq) with 200 mg of Calcein Blue (Sigma, St. Louis, MO) was injected into the ascending aorta within the range of physiological pressures. The heart was excised and all attachments but the left ventricle were removed. The left ventricle was cut into five slices parallel to the atrio-ventricular groove. Each slice was visualized under ultraviolet light; the total area and unstained myocardial surface, defined as the area at risk, were measured. Then the slices were immersed in 200 ml of 1% phosphate-buffered saline (PBS) containing 1% triphenyltetrazolium chloride (TTC) (Sigma) for 40 min at 40°C. Viable myocardial cells containing active nicotinic acid dehydrogenase were stained dark red and dead cells, area of myocardial infarction, remained unstained. Each slice was weighed and the surface area of the infarct was measured. For each slice, the area at risk, the

infarct surface area, and the total area were determined by means of computer-assisted planimetry (Perfect Image version 2.01, Grey Stone).

Histology and immunohistochemistry

Transmural heart pieces of ~10 × 10 mm^2 were cut from the center of the infarct and from a region of nonischemic myocardium chosen in a TTC-positive area. Samples were fixed in 4% formalin in PBS or in ethanol and were embedded in low-melting-point paraffin wax (Paraplast xTra, tissue embedding medium; Oxford, St. Louis, MO). Sections 5-μm thick were cut. Nuclei were stained with Harris' hematoxilin.

Immunohistochemistry was carried out on deparaffinized and rehydrated 5-μm sections. Nonspecific binding was blocked by 1% bovine serum albumin in PBS. Monoclonal antibodies to α-smooth muscle (SM) actin (Rockland, Gilbertsville, PA) and von Willebrand factor (vWF) (DAKO, Trappes, France) were used at a dilution of 1:400 and 1:2000. Incubation with primary antibodies was carried out at room temperature for 2 h in a humidified atmosphere. The unlabeled antibodies were left to react with secondary biotinylated swine multilink (anti-goat, rabbit, and mouse) (DAKO) antibodies at a dilution of 1:100 for 30 min at room temperature. The staining was revealed out with avidin biotin peroxidase complex (Vectastain Elite ABC Kit) and VIP substrate (Vector). Nonimmune swine serum and monoclonal mouse IgG1 to *Aspergillus Niger* glucose oxidase (DAKO) were used as negative reagent controls.

Quantification was performed randomly in a blind operation. For vessel counts, five fields were randomly chosen within the fibrotic areas. In each field, vWF-positive capillaries (<10 μm external diameter) were counted in five randomly chosen unit areas (125 × 125 μm) using a section ocular micrometer (Olympus, Tokyo, Japan) at 40× magnification. α-SM actin-positive microvessels (<25 μm external diameter) were counted in five randomly chosen unit areas (500 × 500 μm) at 10× magnification. In each field, the mean number of vWF-positive and/or α-SM cell actin-positive blood vessels was calculated. Total numbers were extrapolated from these mean values.

Statistical analysis

All data are expressed as means ± SEM. Differences between groups were evaluated by using unpaired *t* tests to compare areas and vessel numbers. Comparisons of repeated measurements between groups were made with repeated-measure ANOVA. Correlations between continuously distributed variables were tested by univariate regression analysis. Differences were considered significant at a *P* value of <0.05.

RESULTS

Results were obtained for seven control pigs and seven RGTA-treated pigs. Throughout the experiment, the following parameters were similar in the two groups: body weight, blood temperature, and major heamodynamic parameters including heart rate, mean arterial pressure, mean pulmonary arterial pressure, right atrial pressure, pulmonary capillary wedge pressure,

cardiac output, systemic vascular resistance index, pulmonary vascular resistance, and left ventricular stroke work index (Table 1). Before and 60 min after ligation, left ventricular ejection fractions were similar in the two groups (Table 2), suggesting similar injuries in each animals of both series. Three weeks after ligation, the ejection fraction had recovered significantly in the RGTA group, but had decreased in the control group (control vs. RGTA: 35.6±3.0 vs. 56.4±4.8%, $P<0.001$, Table 2). Left ventricular end-diastolic volume and end-systolic volume were similar in the two groups at start. However, the systolic ejection volume showed a significant increase in the RGTA group (control vs. RGTA: 27.7±3.7 vs. 51.1±7.0 ml, $P<0.0.1$, Table 2). End-diastolic pressure in the RGTA group was lower than in the control group (control vs. RGTA: 12.3±1.1 vs. 8.7±1.1 mmHg, $P<0.05$), whereas left ventricular weights were similar in the two groups (not shown).

The initial infarction area was only appreciated by the epicardial cyanosis, and was similar in the two groups. As the animals were randomly chosen and presented similar postligation hemodynamic parameters, we assumed that the resulting total infarction area was similar. This area was therefore not measured for 3 weeks when the animals were killed. At this time, the infarct area was 459 ± 74 mm^2 in the RGTA-treated group and 828 ± 12 mm^2 ($P<0.05$) in the control group (Table 3).

The histological aspects of the nonischemic myocardium were identical to that of normal pig heart (not shown). Figure 1 represents histological sections of the central area of infarcted myocardium in tissue from RGTA-treated (*a, b*) and untreated animals (*c, d*) stained with anti-alpha smooth muscle (α SM) (Fig. 1) and von Willebrand Factor (vWF) antibodies (Fig. 2). Large zones of preserved myocytes were detected in RGTA-treated animals (noted M) and absent from controls, where essentially fibrotic tissues were seen (noted F). Background staining of nuclei with Harris hematoxilin revealed a higher nuclear density in the fibrotic control hearts (Fig. 1 *c, d*) than in RGTA-treated animals. Staining of SM cells of the arterioles and vessels with anti-α SM antibodies and of vascular endothelial cells with anti-vWF antibodies visualized vascularization (arrows), which increased significantly in RGTA-treated animals. The mean numbers of vWF-positive capillaries and α SM-positive vessels in five fields were two- to threefold higher in RGTA-treated animals (300 vs. 800, $P<0.01$ for alpha smooth muscle; see Table 3). This difference in the vasculature of the infarcted area in RGTA-treated animals was not only quantitative but also qualitative. An enlargement of a vessel stained with anti-vWF antibodies (no counter staining) is presented in Figure 2*b* (RGTA-treated) and Figure 2*d* (control). Note the higher levels of organization and endothelial cell differentiation in Figure 2*b* than in Figure 2*d*.

DISCUSSION

Our results indicate that RGTA11 changes the healing pattern of the infarcted myocardium, protects large zones of myocytes from degeneration, and favors the development of a more vascularized fibrotic area. Moreover, it reduces the loss of myocardial function with a recovery >80% of the initial LVEF, LVSEV, and LVEDP.

The pig model was selected in our study because *1*) myocardial and coronary anatomies are similar in humans and pigs (17); *2*) the left circumflex artery supplies ~20% of the myocardium,

an anatomical specificity favorable to avoid high mortality (17); and *3*) coronary collaterals are scarce and deliver little blood flow in pigs compared to dogs (18), so the influence of preexisting collateral flow to developing myocardial infarction should be minimized.

As with any experimental model, however, there are weaknesses. First, absolute control of the reproducibility of the ischemia would have needed a larger number of animals and killings of animals postligation. As only 14 pigs were finally enrolled, we had to select them randomly to attenuate the individual variations. Conditions for obtaining a reproducible risk area after coronary occlusion were previously assessed with a first series of five animals. As shown in Table 1, postligation, there were no major differences between RGTA treated and nontreated animals in the heamodynamic parameters, including left ventricular ejection fraction, which dropped by 27% in both groups, suggesting that the injuries were similar. Second, the relevance of the experimental conditions chosen with similar situation in human is difficult to assess. One hour after ligation was chosen for treating with RGTA for practical reasons. Previous reports indicated that 40–60 minutes in acute ischemia would induce a cascade of proteolytic enzyme activation leading to tissue destruction (19, 20). However, no specific studies were done by us in this model, inversely, their are no data on human which would provide information on evolution of the damages of the infarcted muscle after acute ischemia.

The results that we obtained by a single injection of RGTA in the infarcted myocardium recall those observed 1 week after injection of RGTA11 after acute ischemia of the EDL muscle. Indeed, survival of muscle fibers was increased more than twofold, fibrotic area reduced, and vascularization enhanced in the central muscle zone (2). At this stage of our understanding, the mechanisms of RGTA *in vivo* on infarcted myocardium can only be speculative. From *in vitro* studies, we can postulate that RGTA could protect selectively HBGF, among those VEGF and FGF. These factors are vasoactive and enhance microvascularization in chronically ischemic porcine myocardium (21). Ischemic vessels are particularly sensitive to vasodilatation induced by growth factors, probably because of bFGF and VEGF receptor expression induced by chronic ischemia. Reduction of oxygen tension also induces various HBGF, including TGFß molecules and their receptors on endothelial cells (22). We had shown that RGTA can also interact with and protects TGFß (5). Hence, the increased number of vessels in our study could have resulted from protection of these factors by RGTA. Strong support for this hypothesis has recently been obtained (23) in mice deficient in both heparan sulfate-binding isoforms of $VEGF_{164}$ and $VEGF_{188}$ but not in $VEGF_{120}$ (the nonheparin-binding isoform), which showed impaired angiogenesis and ischemic cardiomyopathies. RGTA could indeed protect these forms and, by inhibiting plasmin (10), prevent the formation of $VEGF_{120}$. We therefore propose that RGTA may act as a survival and protective agent through the maintenance and protection of the bioavailability of preexisting and newly synthesized growth factors. These factors are stored in the extracellular matrix through their heparan sulfates. Heparinase is among the first activated enzyme after injury (24) and by cleaving heparan sulfates releases the bound HBGF. Another expected mode of action of RGTA would be to bind to the heparin-binding sites of the extracellular matrix proteins that had become available after the heparanase destruction. By taking the place of the heparan in the matrix, the RGTA is likely to protect the matrix proteins to which it binds. This hypothesis is now been investigated and unpublished data indicate that RGTA binds to collagen, fibronectin, and laminin elastin. Furthermore, labeled RGTA intravenously injected in a noninjured animal is readily eliminated, whereas in an animal after

EDL, crushing remains only in the damaged muscle and is detected until regeneration is completed (Meddahi et al., unpublished data). It is interesting that RGTA11 was shown to inhibit heparinase with a Ki in the nanomolar range (I. Vlodavski and D. Barritault, unpublished results).

By combining HBGF and extracellular matrix protections, RGTA presents an advantage over a simple action triggered by the supply of any single growth factor as for the treatment of patients with ischemic heart, which are now undergoing human trials. Indeed, RGTA polymers are easier and less costly to produce, store, and handle than growth factors. Other RGTA devoid of the potentially carcinogenic benzylamide group are currently been developed as a new class of therapeutic agents to treat ischemia.

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REFERENCES

1. Tardieu M., Gamby C., Avramoglou T., Jozefonvicz J., and Barritault D.(1992) Derivatized Dextrans mimic heparin as stabilizers, potentiators, and protectors of acidic or basic FGF. *J. Cell Physiol.* **150**,194-203

2. Desgranges P., Barbaud C., Caruelle, J. P., Barritault D., and Gautron J. (1999) A substituted Dextran enhances muscle fibers survival and regeneration in ischemic and denervated rat EDL muscle. *FASEB J.* **13**, 761-766

3. Meddahi A., Blanquaert F., Saffar J. L., Colombier M. L., Caruelle J. P., Josefonvicz J., and Barritault D. (1994) New approaches to tissue regeneration and repair. *Pathol. Res. Pract.* **190**, 923-928

4. Blanquaert F., Saffar J. L., Colombier M. L., Carpentier G., Barritault D., and Caruelle J. P. (1995) Heparan-like molecules induce the repair of skull defects. *Bone* **17**, 499-506

5. Meddahi A., Benoit J., Ayoub N., Sézeur A., and Barritault D. (1996) Heparin-like polymers derived from Dextran enhance colonic anastomosis resistance to leakage. *J. Biomed. Mat. Res.* **31**, 293-297

6. Fredj-Reygrobellet D., Hristova D. L., Ettaiche M., Meddahi A., Jozefonwicz J., and Barritault D. (1994) CMDBS, functional analogue of heparan sulfate as a new class of corneal ulcer healing agents. *Ophthalmic Res.***26**, 325-331

7. Gautron J., Kedzia C., Husmann I., and Barritault D. (1995) Injection of a heparan sulfate like substance in a crushed muscle accelerates its regeneration. *C. R. Acad. Sci. III* **318**, 671-676

8. Aamiri A., Mobarek A., Carpentier G., Barritault D., and Gautron J. (1995) Effect of a substituted Dextran on reinnervation during regeneration of adult rat skeletal muscle Effects *C. R. Acad. Sci. III* **318**, 1037-1043

9. Meddahi A., Lemdjabar H., Caruelle J. P., Barritault D., and Hornebeck W. (1996) FGF protection and inhibition of human neutrophil elastase by carboxymethyl benzylamide sulfonate Dextran derivatives. *Int. J. Bio.l Macromol.* **18**, 141-145

10. Meddahi A., Lemdjabar H., Caruelle J. P., Barritault D., and Hornebeck W. (1995) Inhibition by Dextran derivatives of FGF-2 plasmin-mediated degradation. *Biochimie* **77**, 703-706

11. Yanagisawa-Miwa A., Uchida Y., Nakamura F., Tomaru T., Kido H., Kamijo T., Sugimoto T., Kaji K., Utsuyama M., Kurashima C., and Ito H. (1992) Salvage of infarcted myocardium by angiogenic action of basic fibroblast growth factor. *Science* **257**, 1401-1403

12. Harada K., Grossman W., Friedman M., Edelman E. R., Prasad P. V., Keighley C. S., Manning W. J., Sellke F. W., and Simons M. (1994) Basic fibroblast growth factor improves myocardial function in chronically ischemic porcine hearts. *J. Clin. Invest.* **94**, 623-630

13. Banai S., Jaklitsch M. T., Shou M., Lazarous D. F., Scheinowitz M., Biro S., Epstein S. E., and Unger E. F. (1994) Angiogenic-induced enhancement of collateral blood flow to ischemic myocardium by vascular endothelial growth factor in dogs. *Circulation* **89**, 2183-2189

14. Schlaudraff K., Schumacher B., Specht B. U., Seitelberger R., Schlosser V., and Fasol R. (1993) Growth of new coronary vascular structures by angiogenetic growth factors. *Eur. J. Cardiothorac. Surg.* 7, 637-644

15. Uchida Y., Yanagisawa-Miwa A., Nakamura F., Yamada K., Tomaru T., Kimura K., and Morita T. (1995) Angiogenic therapy of acute myocardial infarction by intrapericardial injection of basic fibroblast growth factor and heparin sulfate: An experimental study. *Am. Heart J.* **130**, 1182-1188

16. Mauzac M., and Josefonvicz J. (1984) Anticoagulant activity of Dextrans derivatives. Part I: synthesis and characterization. *Biomaterials* **5**, 301-304

17. White F. C., Roth D. M., and Bloor C. M. (1986) The pig as a model for myocardial ischemia and exercise. *Lab. Anim. Sci.* **36**, 351-356

18. Harken A. H., Simson M. B., Haselgrove J., Wetstein L., Harden III W. R., Barlow C. H. (1981) Early ischemia after complete coronary ligation in the rabbit, dog, pig, and monkey. *Am. J. Physiol.* **241**, H202-H210

19. Schaper W. (1984). Experimental infarcts and the microcirculation. In *Therapeutic Approaches to Myocardial Infarct Size Limitation* (Hearse D. J., and Yellon D. M., eds) pp. 79-86, Raven, New York

20. Simoons M. L., Lubsen J., Serruys P. W., and Hugenholtz P. G. (1987) Thrombolytic therapy for acute coronary thrombosis. *Eur. Heart J.* **8**, 78-87

21. Sellke F. W., Wang S. Y., Stamler A., Lopez J. J., Li J., and Simons M. (1996) Enhanced microvascular relaxations to VEGF and bFGF in chronically ischemic porcine myocardium. *Am. J. Physiol.* **271**, H713-H720

22. Thompson N. L., Bazoberry F., Speir E. H., Cascells W., Ferrans V. J., Flanders K. C., Kondaiah P., Geiser A. G., and Sporn M. B. (1998) Transforming growth factor beta-1 in acute myocardial infarction in rats. *Growth Factors* **1**, 91-99

23. Carmeliet P. (1999) Impaired myocardial angiogenesis and ischemic cardiomyopathy in mice lacking the vascular endothelial growth factor isoforms $VEGF_{164}$ and$VEGF_{188}$. *Nat. Med.***5**, 495-502

24. Vlodavski I., Friedman Y., Elkin M., Aingorn H., Atzmon R., Ishai-Micheli R., Bitan M., Pappo O., Peretz T., Michal I., Spector L., and Pecker I. (1999) Mammalian heparanase: gene cloning, expression and function in tumor progression and metastasis. *Nat. Med.* **5**, 793-802

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Table 1

	CONTROL					RGTA				
	Time					Time				
	baseline	10 min	60 min	90 min	3 weeks	baseline	10 min	60 min	90 min	3 weeks
Heart rate	113±10	113±7	116±10	108±9	110±5	126±10	106±7	108±6	104±7	108±7
MAP	73±4	71±5	68±4	70±5	76±6	86±7	72±3	75±4	84±6	86±10
MPAP	14.4±1.3	16.9±2.5	16.4±1.8	15.7±1.9	15.4±0.9	17.4±0.2	16.9±1.1	19.6±3.5	22.9±4.3	17.7±1.6
RAP	0.9±0.9	3.3±1.1	2.6±0.8	2.1±1.2	1.4±0.8	1.3±1.5	4.0±2.4	4.7±2.3	4.6±2.3	1.9±0.9
PCWP	4.7±1.2	7.0±1.6	7.3±1.1	6.4±1.0.	6.0±0.6	6.7±0.8	9.3±1.3	9.2±1.1	9.0±0.8	5.7±0.6
CO	4.0±0.5	3.5±0.5	3.9±0.5	3.5±0.5	3.7±0.5	4.3±0.6	3.6±0.6	3.2±0.4	3.4±0.4	4.2±0.5
SVRI	1759±333	1851±344	1757±445	2029±513	1824±153	1877±314	1951±430	2099±296	2268±343	1808±236
PVRI	225±37	265±35	192±62	268±64	246±45	208±37	201±40	204±25	298±58	250±41
LVSWI	31.0±4.0	25.7±3.9	26.5±3.8	27.1±4.5	31.7±6.1	34.9±5.7	26.5±3.8	25.3±5.2	27.2±5.7	49.3±4.3

Table 1: Hemodynamic parameters.
MAP: mean arterial pressure; MPAP: mean pulmonary arterial pressure; RAP: right arterial pressure; PCWP: pulmonary capillary wedge pressure (mmHg); CO: cardiac output (l/min); SVRI: systemic vascular resi stance index; PVRI: pulmonary vascular resistance index (dynes.s.cm $^{-5}$); LVSWI: left ventricular stroke work index (g.m/m^2). Data are presented as mean ± SEM.

Table 2

	CONTROL			RGTA		
	Time			Time		
	baseline	Post-ligation	3 weeks after ligation	baseline	Post-ligation	3 weeks after ligation
LVEF	68 ± 2.1	50.0 ± 2.9	35.6 ± 3.0	66.7 ± 3.3	48.3 ± 3.1	56.4 ± 4.8 ***
LVEDV	81.4 ± 4.8	76.7 ± 4.6	86.4 ± 6.4	82.6 ± 10.4	69.6 ± 6.1	93.1 ± 12.4
LVESV	26.0 ± 2.1	38.6 ± 3.3	58.6 ± 4.8	26.9 ± 3.6	36.4 ± 4.0	42.3 ± 8.1
LVSEV	55.9 ± 3.7	38.7 ± 2.8	27.7 ± 3.7	56.3 ± 8.4	33.0 ± 3.6	51.1 ± 7.0 **
LVEDP	8.1 ± 0.7	12.9 ± 1.3	12.3 ± 1.1	6.6 ± 1.3	14.7 ± 2.6	8.7 ± 1.1 *

Table 2: Physiological parameters.
LVEF: left ventricular ejection fraction (%); LVEDV: left ventricular end-diastolic volume; LVESV: left ventricular end-systomlic volume; LVSEV: left ventricular systolic ejection volume (ml); LVEDP: left ventricular end-diastolic pressure (mmHg). Data are presented as mean ± SEM. *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$ compared with control.

Table 3

	control	RGTA	p
Infarction size [mm^2]	459±74	828±12	<0.05
Number of vW+ cells	120	220	<00.5
Number of αSM+ cells	300	800	<0.01

Table 3: Histological parameters at 3 weeks.

Data are presented as means ± SEM. VW + cells: cells stained with anti-von Willbrand antibodies. αSM+ cells: cells stained with anti-alpha smooth muscle actin antibodies. For both immunostainings, values were obtained in five randomly chosen fields of 2500 μm^2. Differences between groups were evaluated by using an unpaired t test.

Fig. 1

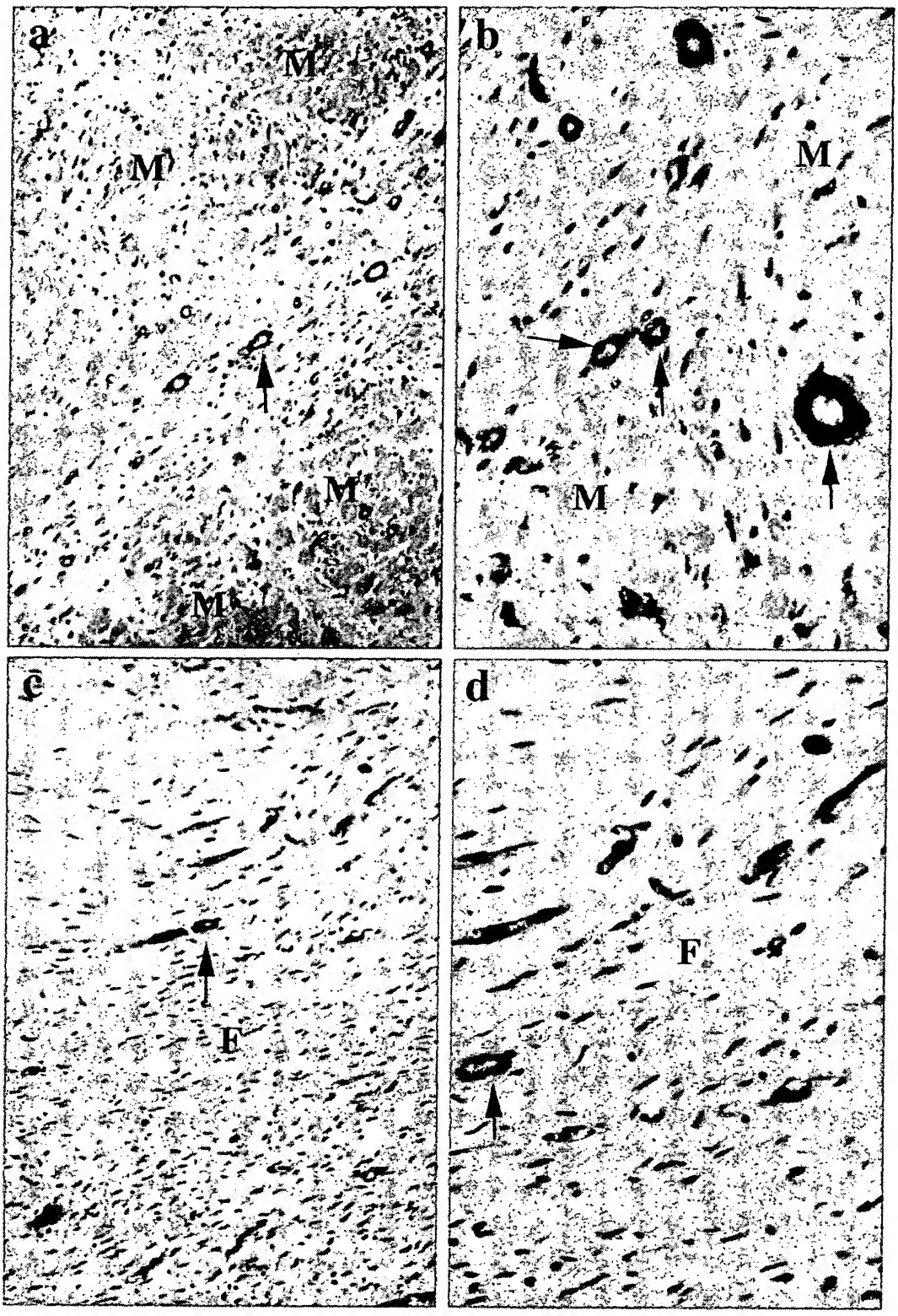

Figure 1. Histological sections of infarcted area stained with anti -αsmooth muscle actin antibodies. a and b are from RGTA-treated hearts; c and d are from saline-treated hearts. Arrows point to the positively stained arterioles and vessels. F indicates a fibrotic area; M indicates remaining myocytes. Nuclei were stained with Harris hematoxylin. Enlargements are: a, c x150 and b, d x375.

Fig. 2

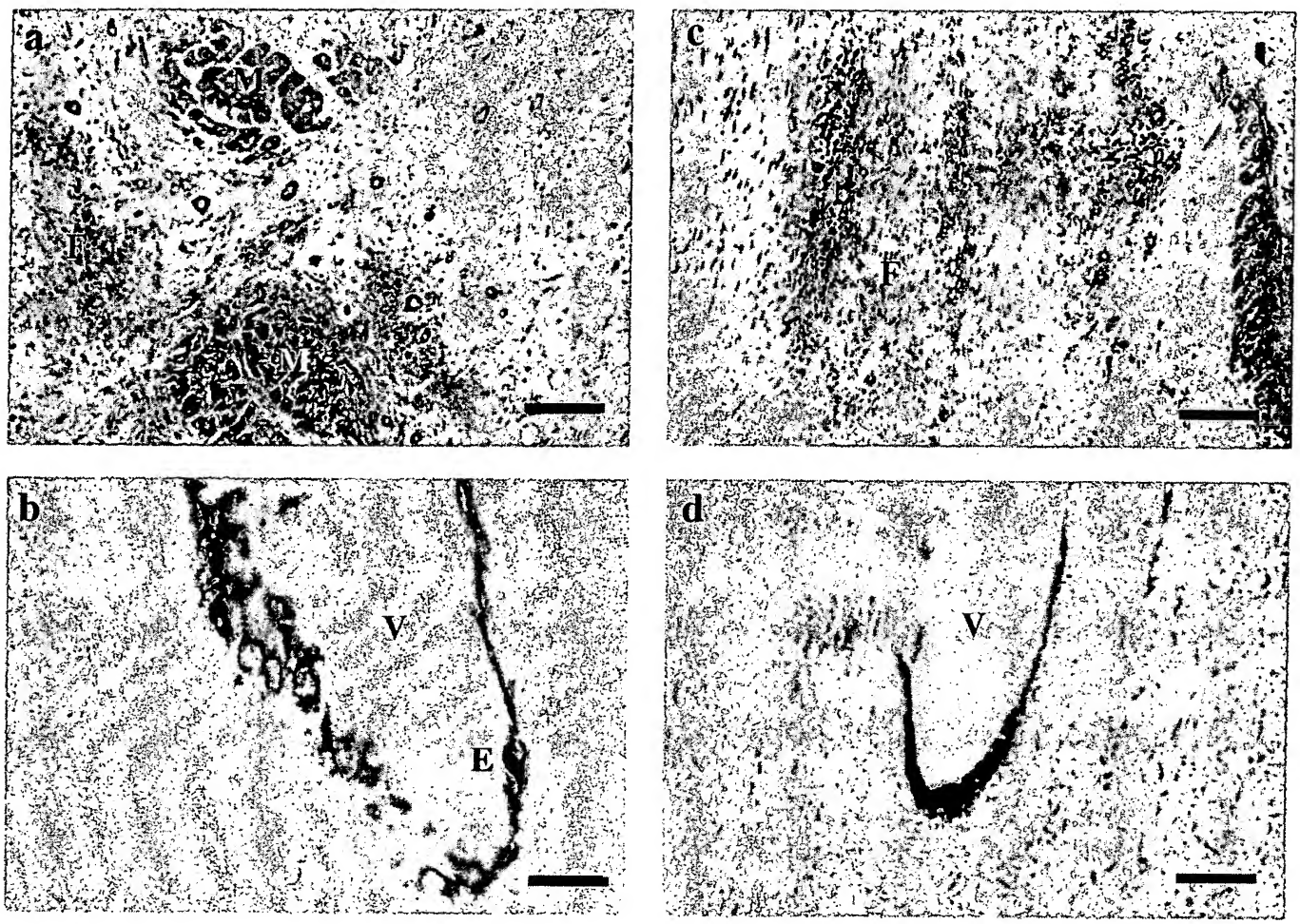

Figure 2. Histological sections of an infarcted area stained with anti-von Willbrand(vW) antibodies. Same as for fig.1. E = endothelial cells. Enlargements are: a, c x100, bar=100 μm; b, d x400, bar = 25μm.